

PESTICIDE AND INDUSTRIAL CHEMICAL RESIDUES

Optimization of Experimental Conditions for the Supercritical Carbon Dioxide Extraction of Pesticide Residues from Grains

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The efficiency of a supercritical carbon dioxide extraction system was investigated for the extraction at different pressures and temperatures of fortified grain samples containing organochlorine, organophosphorus, and organonitrogen pesticides from grain matrixes. The extractor unit was constructed using a gas booster pump and a Florisil sorbent trap for extracting and isolating the residues of interest. Using 20 g samples, extractions were performed between 40° and 80° C with pressures from 2000 to 10 000 psig. In most cases, pesticide recoveries exceeding 80% were recorded over the above range of temperature and pressure. Excellent recoveries and precision were recorded for an incurred methyl chlorpyrifos residue at the 0.043 ppm level. An improved liquid chromatographic assay was also developed for the analysis of carbofuran in grain samples.

The analysis of pesticide residues in grains intended for human consumption (1) or in the production of fermentation-derived chemicals (2) is of increasing importance because of tolerance and revised-action levels developed by regulatory agencies. In addition, regulatory agencies (3) are increasing scrutiny of traditional pesticide sample workup and analysis methodologies because of the disposal problems and environmental impact of the organic solvents used in such methods. Supercritical fluid extraction (SFE) is a viable alternative to traditional organic solvent-based methods used in the analysis of pesticide residues (4–6).

Several researchers (7–9) demonstrated the efficacy of SFE of selected pesticides from grain and crop matrixes. However,

optimal extraction conditions applicable to many types of pesticides need to be identified so that the extraction procedure can be used as a multiresidue analysis screening method.

Theoretical schemes were applied by several investigators (10, 11) in an attempt to optimize extraction conditions for SFE. Currently, the most effective way to optimize the extraction step for multiresidue analysis is to run a series of experiments that carefully delineate factors contributing to optimum pesticide recoveries. The present study was undertaken to define optimum conditions for the extraction of several classes of pesticides commonly found in wheat, including methoxychlor, chlorpyrifos, dieldrin, malathion, pirimiphos-methyl, dimethoate, parathion-methyl, and carbofuran.

Samples obtained from the Federal Grain Inspection Service (FGIS) were fortified at 2 spiking levels (5.0 and 0.1 ppm) and extracted by using supercritical carbon dioxide (SC-CO₂) at combinations of 3 different pressures and temperatures. The extracted analytes were collected after decompression of SC-CO₂ on a sorbent-filled tube containing Florisil. Conventional elution solvents (12) were applied to desorb the pesticides from the sorbent cartridge before additional sample cleanup and/or direct analysis by gas chromatography (GC) or liquid chromatography (LC).

Experimental

Apparatus and Reagents

The extraction apparatus was similar in construction and operating principle to a previously described device (13). Modifications consisted of the insertion of an alumina cleanup column to purify the extraction fluid before the compressor, the placement of the extractor vessel in a Bendix 2600 GC oven, and the installation of Florisil-filled trap after the micrometering valve (Figure 1). One of 2 gas booster compressors were used depending on the desired extraction pressure.

(a) *Compressors*.—Models AGT-62/152 or AGC-30 (Haskel Engineering Corp., Burbank, CA) were used to generate the CO₂ pressure required for the extractions. The Model AGC-30 was used for the extractions at 2000 psi, because it provided better pressure control in this region.

(b) *Alumina cleanup column*.—A column for cleaning up the extraction fluid was constructed from 316 SS tubing (Part No. 15-009, Autoclave Engineers, Erie, PA), pressure rated to

Received October 1, 1992. Accepted December 24, 1992.

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Presented at the 105th AOAC International Annual Meeting, August 12–15, 1991, at Phoenix, AZ.

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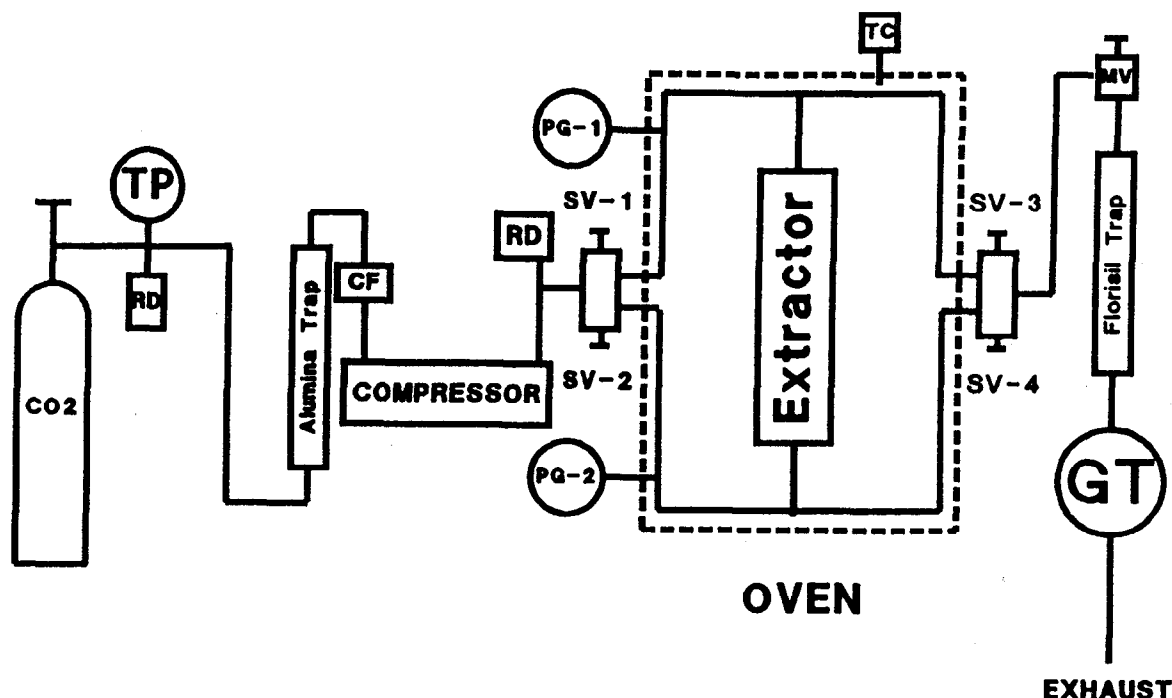


Figure 1. Supercritical fluid extraction apparatus with sorbent trap option: TP = tank pressure, RD = rupture disk, CF = check valve and filter, PG = pressure gauge, SV = switching valves, TC = thermocouple, MV = micrometering valve, and GT = gas totalizer.

76 MPa (11 000 psi) at room temperature, with dimensions of 56×1.75 cm id, filled with Alumina C held in place by glass wool plugs.

(c) *Florisil trap*.—A 316 SS column, 30.4×0.95 cm id, containing activated Florisil held in place by glass wool plugs was used as the pesticide trap.

(d) *Concentrator system*.—A miniature concentrator system consisting of a distilling trap adapter (No. 5226, Ace Glass, Inc., Vineland, NJ) and a micro evaporative concentrator (No. 6709, Ace Glass, Inc.) was constructed.

(e) *Gel permeation chromatograph*.—A gel permeation chromatographic (GPC) system, Auto-Prep 1002B (ABC Laboratories, Inc., Columbia, MO), equipped with a 30×2.5 cm id column (ABC Laboratories) slurry packed with 33 g Bio-Beads SX-3 resin (200–400 mesh, Bio-Rad Laboratories, Richmond, CA) and compressed to a bed length of ca 20 cm, was used for further cleanup of the extracted fractions. The elution solvent was methylene chloride–hexane (50 + 50, v/v) pumped at a flow rate of 5.0 mL/min at an operating pressure range of 8 to 11 psig. The GPC system was set up to execute a 12 min dump, 16 min collect, and 0 min wash cycle. The sample loading filter consisted of a $5.0 \mu\text{m}$ prepdisc membrane filter (Bio-Rad).

(f) *Gas chromatograph 1*.—A Varian Model 3600 GC system, equipped with an Ni-63 electron capture detection (ECD) system, a flame photometric detection (FPD) system using a phosphorus-specific filter (526 nm), a septum-equipped programmable injector (SPI), a packed-column injector, and an 8100 autosampler, was used for the analysis of specific pesticides. The packed-column injector was modified for the direct flash vaporization injection onto a wide-bore fused silica col-

umn as previously described (14). A $2 \text{ m} \times 0.53$ mm id, presilanized, uncoated fused silica retention gap (No. 1602535, J&W Scientific, Inc., Folsom, CA) was connected to the modified inlet through a universal glass connector (No. 2-0479, Supelco, Inc., Bellefonte, PA) to a $15 \text{ m} \times 0.53$ mm id, $1.0 \mu\text{m}$ /min film thickness, DB-17 fused silica column (No. 125-1712, J&W Scientific, Inc.). A $10 \text{ m} \times 0.53$ mm id, $1.0 \mu\text{m}$ film thickness, DB-1301 fused silica column (No. 125-1312, J&W Scientific, Inc.) was connected to a DB-17 column through a universal glass connector and then to a makeup gas fitting (No. 103462, Scientific Glass Engineering, Austin, TX) for connection to the FPD system. A 2 m retention gap was then connected to the SPI inlet and connected to a universal glass connector that was fastened to a $15 \text{ m} \times 0.53$ mm id, $1.5 \mu\text{m}$ film thickness, DB-5 fused silica column (No. 125-5012, J&W Scientific, Inc.). The DB-5 column was then connected to the makeup gas fitting for the ECD system. Flow rates of ca 10 and 15 mL/min of helium, respectively, were used for the DB-5 and DB-17+DB-1301 columns. A makeup gas flow rate of 15 mL/min nitrogen was used for each detector. The following temperature parameters were used for ECD in Method 1: SPI inlet, initial 60°C with 0 min hold, programmed at $275^\circ\text{C}/\text{min}$ to 240°C final temperature with 15 min hold; ECD system, 300°C ; column, 100°C with 1 min hold, programmed at $10^\circ\text{C}/\text{min}$ to 250°C final temperature with 6 min hold. The following temperature parameters were used for FPD in Method 2: inlet, 220°C ; FPD system, 300°C ; column, 150°C with 1 min hold, programmed at $5^\circ\text{C}/\text{min}$ to 200°C final temperature with 5 min hold.

(g) *Gas chromatograph 2*.—A Tracor Model 540 GLC equipped with a Hall electrolytic conductivity detector

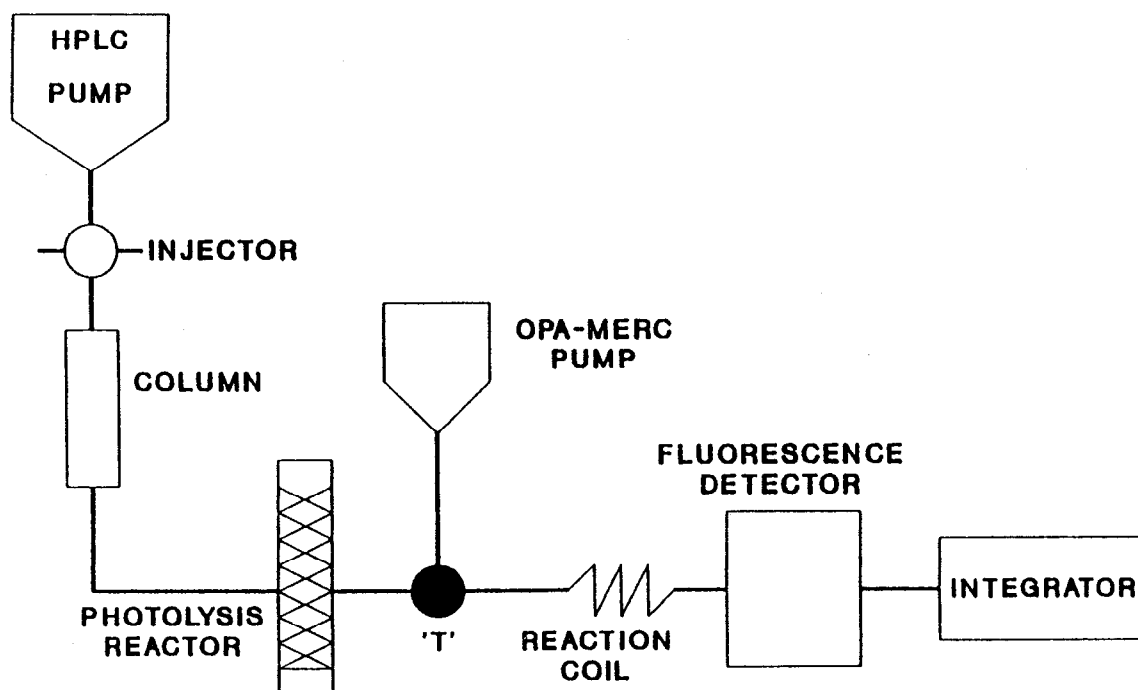


Figure 2. LC apparatus for carbofuran determination.

(HECD) was also used for pesticide analysis after SFE. A 2 m \times 0.53 mm retention gap was connected to the modified packed column inlet as noted above and then through a universal glass connector to a 30 m \times 0.53 mm id, 1.5 μ m film thickness, DB-1 fused silica column (No. 125-1012, J&W Scientific, Inc.). The DB-1 column was connected to the HECD without makeup gas. Hydrogen was used as the carrier gas at a flow rate of ca 30 mL/min. Reaction gas flow rate for the furnace was 50 mL/min hydrogen. Solvent flow rate for the cell was 0.4 mL/min *n*-propanol. The following temperature programming parameters were used: inlet, 220°C; detector base, 260°C; column, 200°C; furnace, 900°C.

(h) *Liquid chromatograph*.—The basic schematic for the LC system used for the analysis of carbofuran in the extracts is shown in Figure 2. This is a modification of a previously described system (15) consisting of an injector, guard column, column, and column oven. Specific chromatographic parameters used are as follows: an Adsorbosphere C-8 column packed with 5 μ m packing in a 150 \times 4.6 mm cartridge, along with a Adsorbosphere C-8 guard column. Injections were 15 μ L into an acetonitrile–water (40 + 60) mobile phase pumped through the column at a flow rate of 1.0 mL/min. The column temperature was 40°C.

The postcolumn photolysis unit consisted of a Model 80-1178-01 BHK Lamp, 17 cm \times 9 mm (BHK, Inc., Pomona, CA), with a Model 90-001-01 power supply (BHK Inc.). The Teflon sleeve surrounding the photodegradation lamp was prepared by weaving 3.7 m \times 0.5 mm id \times 1/16 in. od Teflon tubing into a 3 dimensional coil. Alternatively, one may purchase a 10 ft \times 0.5 mm id commercial delay coil (Part No. 5-9206, Supelco, Inc., Bellefonte, PA). The coiled Teflon tubing was placed over the lamp and connected at one end to the column

outlet and at the other end to the mixing tee, which was a 10.25 mm id \times 1/16 in. od fitting (No. ZT1C, Valco Instruments, Inc., Houston, TX). A low flow rate pump (Model 396-31, LDC/Milton Roy, Riviera Beach, FL) was used for the addition of *o*-phthalaldehyde–mercaptoethanol (OPA–MERC). The reaction coil consisted of 3.0 m \times 0.3 mm id. Teflon tubing and was made into 3 dimensional coil or purchased as a delay coil from Supelco, as noted above. A Shimadzu RF 503 fluorescence detector (Shimadzu, Inc., Columbia, MD), or equivalent, was used to detect the carbofuran.

(i) *Carbon dioxide*.—Welding grade CO₂ (National Welding Supply Co., Bloomington, IL) was used for all of the extractions. Impurities in the CO₂ were removed by the use of an in-line trap filled with Alumina C sorbent.

(j) *Alumina C*.—1 kg (Part No. 02103-99, Universal Scientific Inc., Atlanta, GA) was heated to 230°C for 1 h to achieve maximum activity.

(k) *Florisil*.—A 60/80 mesh fraction of PR grade Florisil (Floridin Co., Berkely Springs, WV) was used for trapping the extracted analytes. The sorbent activity was determined as described in PAM I, Section 121.3.

(l) *Solvents*.—Pesticide or LC grades of the following solvents were used in this study: ethyl acetate, acetone, hexane, methylene chloride, and acetonitrile. Milli-Q reagent grade water (Millipore Corp., Bedford, MA) was used throughout the experiments.

(m) *LC reagents*.—Derivatization and detection agents: OPA and MERC, reagent grade (Sigma Chemical Co., St. Louis, MO). OPA–MERC reagent was prepared by dissolving 75–80 mg OPA in 2 mL methanol and then adding this to 500 mL 0.01M pH 10.5 borate buffer. The solution is then

mixed, 0.5 mL MERC is added, and the solution is mixed again.

(n) *Pesticide standards.*—Pesticide standards used in spiking the wheat samples were obtained from the U.S. Environmental Protection Agency (EPA), Analytical Chemistry Section, Beltsville, MD, or the U.S. Department of Interior, Fish Wildlife Research Center, Chemistry Section, Laurel, MD. Analytical standards were prepared using pesticides obtained from EPA, Pesticide and Industrial Chemicals Repository, Research Triangle Park, NC. All samples and standards were filtered with 0.22 $\mu\text{L}/\text{min}$ nylon polypropylene-encased 13 mm id filters (Alltech Associates, Inc., Deerfield, IL) before LC analysis.

Preparation and Spiking of Grain Samples

Approximately 2500 g of a 5000 g wheat sample was ground on a FN 3100 mill equipped with a 1 mm screen. Forty 50 g samples were then weighed into individual containers for pesticide fortification. Spiking solutions for each pesticide were prepared by weighing 100 mg of each pesticide in a beaker, transferring the pesticide into a 100 mL volumetric flask using ethyl acetate, and diluting to the mark to yield a stock solution of 1 $\mu\text{g}/\mu\text{L}$.

The above solution was used to prepare spiked samples at the 5 and 0.1 ppm level. For the 5 ppm spikes, a 50 g wheat sample was spiked with 250 μL stock solution using a Hamilton No. 1725 gas-tight 250 μL syringe (Hamilton Co., Reno, NV). The open glass bottle was then placed in a hood for 5–6 min until the ethyl acetate was evaporated. The bottle was then capped with an aluminum foil-lined cover. For preparing the 0.1 ppm spikes, the stock solution was diluted 1:10. A 50 μL aliquot of this solution was sampled using a Hamilton No. 805, gas-tight 50 μL syringe and added to a 50 g wheat sample in a glass bottle. The 0.1 ppm spiked wheat samples were then placed in a hood for 2–3 min to let the ethyl acetate evaporate. The ground wheat samples were then thoroughly mixed after fortification and again when sampling for SFE.

Extraction Procedure

A wheat sample (20 g) was weighed into a 250 mL beaker and then poured through a small funnel into the extraction vessel that contained a glass wool plug at the bottom. After filling the extraction vessel with the wheat sample, another glass wool plug was placed on top of the sample after the plug was used to remove any additional sample adhering to the walls of the beaker or funnel. The extraction vessel was then assembled and placed in the oven (Figure 1). Extraction pressure and temperature were set at the desired values and the CO_2 flow commenced. The CO_2 flow rate was set to ca 5 L/min (decompressed flow) as measured under ambient conditions on a dry test meter. Approximately 150 L CO_2 was used to complete the extractions at 2000 psi, whereas 100 L CO_2 was used for the 5000 and 10 000 psi extractions. The extracted analytes were collected on the described Florisil trap after the SC-CO_2 was decompressed. Acetone (50 mL) was used to elute the adsorbed pesticides from the Florisil bed at ambient temperature, and this extract was pooled with a 10 mL acetone rinse of the

micrometering valve (MV in Figure 1). Typical extraction times were 20–30 min.

Sample Cleanup and Analysis

The 20 g wheat samples fortified at 5 ppm were analyzed by diluting the SFE extract to 100 mL in acetone. A 4 mL portion of this solution was diluted to 10 mL with acetone. The organophosphorus pesticides in this solution were quantitated with external standards using FPD. The organochlorine pesticides were quantitated with external standards using ECD and/or the HECD. Chlorinated and organophosphate pesticide standards diluted from 1.0 mg/mL stock solution in acetone to a final concentration of 0.4 $\mu\text{g}/\text{mL}$ were used for this purpose.

The 20 g wheat samples fortified at 0.1 ppm were analyzed by diluting the SFE extract to 100 mL in acetone. A 50 mL portion of this solution was pipetted into a Kuderna-Danish evaporator (250 mL) with a 10 mL graduated concentrator tip. The sample was then evaporated to <5 mL using a Snyder column and steam bath. *n*-Hexane (50 mL) was then added through the Snyder column and the eluate was reconcentrated. This sample eluate was then concentrated to a small volume (<3 mL) with a distilling trap adapter and micro-evaporative concentrator. The volume was adjusted to 5 mL with *n*-hexane, diluted to 10 mL with methylene chloride, and thoroughly mixed. Then, 5 mL of each sample was cleaned-up by the GPC method described by Hopper (16). The sample eluate was transferred to a Kuderna-Danish (250 mL) with a 4 mL graduated concentrator tube and concentrated to a small volume (<3 mL). The eluate was further concentrated (<1 mL) as previously described and diluted to a final volume of 2 mL with acetone. The organophosphorus and organochlorine pesticides were quantitated by GC as described in *Apparatus and Reagents*.

Also, duplicate 20 g wheat samples fortified at 5 and 0.1 ppm were extracted in duplicate by the conventional 35% water-acetonitrile procedure (17). Sample extracts were partitioned with 100 mL petroleum ether and the recovered volumes were recorded. All sample extracts were then diluted to 100 mL with petroleum ether. The organophosphorus and organochlorine pesticides contained in the 5 ppm fortifications were quantitated, without further dilution, by GC as described in *Apparatus and Reagents*. The sample extracts from the 0.1 ppm fortifications were analyzed by the procedure as described in *Apparatus and Reagents*, except that sample eluates were diluted to a final volume of 1 mL in acetone. Wheat sample blanks were also analyzed during each procedure to check for interfering responses. Methyl chlorpyrifos was found as an incurred residue in the wheat at a sub-part-per-million level.

For the determination of carbofuran by LC, the extract from the 5.0 ppm spiked wheat sample was diluted to 100 mL with acetone. Two milliliters of this solution was then pipetted into a 5 mL graduated cylinder and evaporated to dryness with nitrogen at 40°C. The resulting residue was dissolved in acetonitrile–water (1 + 1) and filtered for LC before analysis.

For the 0.1 ppm spiked wheat samples, the extract representing 20 g of SFE-extracted wheat was diluted to 100 mL with acetone. A pipet was then used to transfer 25 mL of the above solution to a 250 mL Kuderna-Danish evaporator. Hex-

Table 1. Pesticide recoveries (%) at 2000 psi extraction pressure as a function of temperature (150 L CO₂)

	Spike level, ppm	40°C		60°C		80°C	
		A	B	A	B	A	B
Dimethoate	5.0	75	76	74	75	32	26
	0.1	91	92	82	86	53	63
Methyl parathion	5.0	76	71	78	71	64	65
	0.1	103	100	87	89	84	87
Pirimiphos-methyl	5.0	93	82	102	93	90	89
	0.1	106	102	99	99	93	94
Chlorpyrifos	5.0	95	84	105	97	93	89
	0.1	104	97	97	96	95	97
Malathion	5.0	81	78	84	78	74	72
	0.1	111	108	103	99	95	94
Dieldrin	5.0	102	89	97	96	93	92
	0.1	98	87	84	85	90	90
Methoxychlor	5.0	104	88	99	94	74	68
	0.1	110	90	78	81	75	75
Carbofuran	5.0	82	74	80	81	77	73
	0.1	95	90	87	90	85	81

ane (30 mL) was added, and the solution was evaporated on a steam bath to 3 mL; an additional 30 mL portion of hexane was added during the evaporation. The cooled liquid was transferred to a 15 mL centrifuge tube and evaporated to dryness using a stream of nitrogen at 40°C. Two milliliters acetonitrile–water (1 + 1) and 0.5 mL hexane previously saturated with acetonitrile–water (1 + 1) were added. The mixture was shaken 30 s and centrifuged at 1000 rpm for several minutes to clarify the layers. The lower aqueous layer was withdrawn and filtered before LC analysis.

The apparatus shown in Figure 2 was used to assay the wheat sample extracts. Sample chromatograms were compared with a chromatogram obtained from the analysis of a standard solution of carbofuran. A 1.0 mg/mL carbofuran stock solution prepared in methanol was used to prepare 2 working standards: 1 µg/mL, used to assay the 5 ppm spiked samples, and 0.25 µg/mL, used to assay the 0.1 ppm fortified wheats.

Results and Discussion

Extractions were performed at 3 different temperatures and 2 fortification levels in the wheat. Experiments were run using all possible combinations of the above variables. Duplicate runs were made under each experimental condition, for a total of 36 extractions.

Some experimental optimization was done before the initiation of the above extractions. These experiments were run to determine a suitable extraction flow rate, minimize the time of extraction, ascertain the effect of the total volume of extraction

fluid on pesticide recovery, and to determine the required amount of trapping sorbent to avoid breakthrough of the analyte off the sorbent bed. Extraction times of 20–30 min and a CO₂ flow rate of 5 L/min at all extraction pressures and temperatures yielded high analyte recoveries. Experiments using dual Florisil traps (5 and 10 g each) aligned in series indicated that a single trap filled with 5 g Florisil was adequate to trap the pesticides under the above conditions.

Results for SFE of wheat samples at 3 extraction pressures are summarized in Tables 1–3. Listed within each table are the pesticide recoveries at the 3 designated temperatures: 40, 60, and 80°C. Recoveries at both fortification levels are listed side by side for comparison. The letters A and B designate percent recovery value for each of 2 separate extractions under identical conditions of the same spiked wheat sample.

The overall extraction results tabulated in Tables 1–3 are very encouraging. With the exception of a few extractions, pesticide recoveries in most cases were >80%. Tables 1–3 also reveal that somewhat higher recoveries were achieved when extracting at 5000 and 10 000 psi as opposed to 2000 psi, regardless of the extraction temperature. At the 2000 psi extraction pressure, recoveries from the extraction of the 0.1 ppm spiked wheats are higher than those obtained from extracting the 5.0 ppm fortifications. This trend is not evident at the 5000 and 10 000 psi extraction conditions.

The agreement between duplicate extractions at the same fortification levels under identical extraction conditions was good (Tables 1–3). Although not shown with the data in Tables 1–3, the appearance of an incurred residue of methyl chlorpyri-

Table 2. Pesticide recoveries (%) at 5000 psi extraction pressure as a function of temperature (100 L CO₂)

	Spike level, ppm	40°C		60°C		80°C	
		A	B	A	B	A	B
Dimethoate	5.0	86	96	84	95	78	94
	0.1	88	87	82	101	77	84
Methyl parathion	5.0	97	100	95	104	88	98
	0.1	89	89	92	103	91	93
Pirimiphos-methyl	5.0	95	100	98	104	92	100
	0.1	96	95	101	108	99	100
Chlorpyrifos	5.0	92	102	98	107	98	102
	0.1	97	97	105	113	99	101
Malathion	5.0	102	113	104	116	111	109
	0.1	93	95	102	109	96	97
Dieldrin	5.0	90	91	91	91	92	98
	0.1	95	91	104	104	93	91
Methoxychlor	5.0	96	99	91	98	96	101
	0.1	94	94	85	107	97	103
Carbofuran	5.0	93	99	86	86	93	102
	0.1	89	97	97	98	92	95

fos in the wheat allowed an estimate to be made of the reproducibility of the above SFE technique. The level of this incurred residue was determined 18 times when the 0.1 ppm spiked wheat samples were extracted. The average value for the incurred residue was 0.040 ± 0.003 ppm, with a relative standard deviation of 7.92%. These values are more representative of the true precision afforded by the SFE technique and are excellent considering the sub-parts-per-million level of the incurred residue.

SFE results for wheat samples and traditional water-acetonitrile extraction method (17) results for wheat samples are compared by the data in Table 4 and results tabulated in Tables 1-3. The data show that SC-CO₂ extraction is equivalent to the liquid solvent extraction procedure for the listed pesticides. Dimethoate and carbofuran were not recovered using the PAM procedure; however, extraction with SC-CO₂ yields excellent recoveries for both pesticides at 5000 and 10 000 psi and the chosen extraction temperatures. Note that the incurred methyl chlorpyrifos residue, determined upon analysis of the 0.1 ppm spiked wheats, is in excellent agreement with the results obtained by SFE.

The extractions performed at 2000 psi on 5 ppm spiked wheat samples (Table 1) yielded lower recoveries (70-80%) for dimethoate, carbofuran, malathion, and methyl parathion with respect to the other 4 pesticides. At 40 and 80°C, pesticide recoveries dropped relative to recoveries obtained at 5000 psi (Table 2). Dimethoate, in particular, could only be recovered at a 29% level at 2000 psi and 80°C. The above trends are not as prevalent for the 0.1 ppm spiked wheat samples extracted at

2000 psi; recoveries were above 80% for all of the pesticides, except dimethoate and methoxychlor at 80°C. The reason for this subtle difference in pesticide recoveries at the 0.1 and 5.0 ppm levels is not apparent; however, the difference may be related to the need for additional CO₂ or higher pressure to enhance the recovery of the larger amount of pesticide at the 5.0 ppm level.

Recovery results in Tables 2 and 3 at the 5000 and 10 000 psi pressure levels are excellent, averaging between 80 and 110% for all of the pesticides studied over the entire temperature range. The lowest extraction temperature possible should be used to avoid thermal degradation of the analytes. In addition, using the lowest pressure possible reduces the costs associated with high fluid compression and pressure ratings on the equipment.

Lower extraction pressures also minimize the amount of co-extracted lipid matter carried along with the target analytes. Wheat contains about 10% oil, which can potentially be extracted under the conditions used in this study. Less than 20% of this oil is extracted at 10 000 psi and 80°C (18). Extractions performed on the 5.0 ppm fortified samples also had appreciable amounts of coextracted lipid material; however, these extracts required no further sample cleanup, because the extracts could be directly diluted and injected onto the described chromatographic instrumentation at levels that were not deleterious to chromatographic efficiency or detection.

In summary, the above study shows that SFE coupled with sorbent trapping of the target analytes is a viable technique for the extraction of pesticides from grain matrixes. Some caution

Table 3. Pesticide recoveries (%) at 10 000 psi extraction pressure as a function of temperature (100 L CO₂)

	Spike level, ppm	40°C		60°C		80°C	
		A	B	A	B	A	B
Dimethoate	5.0	96	82	84	80	94	101
	0.1	79	79	75	101	87	89
Methyl parathion	5.0	107	94	97	99	104	111
	0.1	81	80	89	106	90	94
Pirimiphos-methyl	5.0	104	93	100	108	115	113
	0.1	89	82	95	111	101	101
Chlorpyrifos	5.0	110	96	93	97	111	108
	0.1	87	88	95	111	101	104
Malathion	5.0	99	96	95	101	113	113
	0.1	85	81	96	115	97	96
Dieldrin	5.0	90	91	92	88	90	94
	0.1	77	84	101	107	103	88
Methoxychlor	5.0	92	96	97	92	89	99
	0.1	76	81	90	107	90	105
Carbofuran	5.0	94	99	93	86	95	100
	0.1	84	86	81	99	88	89

should be exercised, however, because, with the exception of the methyl chlorpyrifos, all of the reported recovery data are on fortified samples. SFE of incurred residues is preferred to SFE of spiked samples whenever possible. The technique can be translated onto commercial instrumentation, and many of the steps can be automated. In addition, desorption of the analytes from the Florisil trap may be possible by using SC-CO₂, thereby further reducing the use of organic solvents even further in the described procedure. An integrated extraction/cleanup method for pesticide residue analysis was recently described by France et al. (19). Additional selectivity for mul-

ti-residue analysis can be achieved by varying the collection sorbent, thereby fractionating individual pesticide classes and coextracted moieties.

Acknowledgments

We would like to thank Clifford Watson and Don Koeltzow, FGIS, Kansas City, MO, and Wilda Martinez, National Program Staff, U.S. Department of Agriculture, Agricultural Research Service, Washington, DC, for facilitating the above study. The assistance of Rafael Sarmiento, FGIS, Washington, DC, in providing the fortified grain samples is gratefully acknowledged.

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Table 4. Pesticide recoveries (%) from the PAM, Vol. 1, sec. 212.13C, extraction procedure

	5.0 ppm		0.1 ppm	
	A	B	A	B
Dimethoate	NF ^a	NF	NF	NF
Parathion-methyl	98	99	101	97
Pirimiphos-methyl	103	104	100	91
Chlorpyrifos	100	101	94	93
Malathion	100	99	105	116
Dieldrin	84	89	106	108
Methoxychlor	98	101	90	89
Carbofuran	NF	NF	NF	NF
Chlorpyrifos-methyl ^b	—	—	0.040	0.039

^a NF = Not found.

^b Incurred residue in parts per million.

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Supplied by U.S. Dept. of Agriculture
National Center for Agricultural
Utilization Research, Peoria, Illinois